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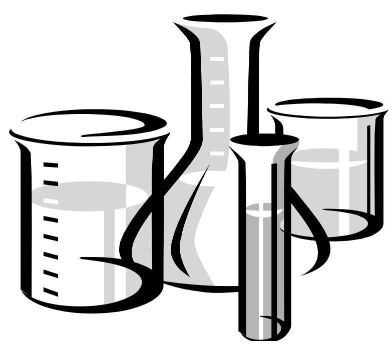
Product Performance Test Guidelines

OCSPP 810.2700: Products with Prion-Related Claims



NOTICE

This guideline is one of a series of test guidelines established by the United States Environmental Protection Agency's Office of Chemical Safety and Pollution Prevention (OCSPP) for use in testing pesticides and chemical substances to develop data for submission to the Agency under the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601, et seq.), the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (7 U.S.C. 136, et seq.), and section 408 of the Federal Food, Drug and Cosmetic Act (FFDCA) (21 U.S.C. 346a). Prior to April 22, 2010, OCSPP was known as the Office of Prevention, Pesticides and Toxic Substances (OPPTS). To distinguish these guidelines from guidelines issued by other organizations, the numbering convention adopted in 1994 specifically included OPPTS as part of the guideline's number. Any test guidelines developed after April 22, 2010 will use the new acronym (OCSPP) in their title.

The OCSPP harmonized test guidelines serve as a compendium of accepted scientific methodologies and protocols that are intended to provide data to inform regulatory decisions under TSCA, FIFRA, and/or FFDCA. This document provides guidance for conducting the test, and is also used by EPA, the public, and the companies that are subject to data submission requirements under TSCA, FIFRA, and/or the FFDCA. As a guidance document, these guidelines are not binding on either EPA or any outside parties, and the EPA may depart from the guidelines where circumstances warrant and without prior notice. At places in this guidance, the Agency uses the word "should." In this guidance, the use of "should" with regard to an action means that the action is recommended rather than mandatory. The procedures contained in this guideline are strongly recommended for generating the data that are the subject of the guideline, but EPA recognizes that departures may be appropriate in specific situations.You may propose alternatives to the recommendations described in these guidelines, and the Agency will assess them for appropriateness on a case-by-case basis.

For additional information about these test guidelines and to access these guidelines electronically, please go to http://www.epa.gov/ocspp and select "Test Methods & Guidelines" on the navigation menu. You may also access the guidelines in http://www.regulations.gov grouped by Series under Docket ID #s: EPA-HQ-OPPT-2009-0150 through EPA-HQ-OPPT-2009-0159, and EPA-HQ-OPPT-2009-0576.

OCSPP 810.2700: Products with Prion-Related Claims.

(a) **Scope**—

(1) **Applicability.** This guideline describes test methods that EPA believes will generally satisfy certain testing requirements of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*), and the Federal Food, Drug, and Cosmetic Act (FFDCA) (21 U.S.C., 301 *et seq.*).

(2) **Background.** *Prion* ("**pr**oteinaceous **in**fectious particle") is a term often used to designate an infectious agent that causes progressive degenerative diseases of the central nervous system, which are collectively called the transmissible spongiform encephalopathies (TSEs or prion diseases), including scrapie, chronic wasting disease (CWD), bovine spongiform encephalopathy (BSE), and various forms of Creutzfeldt-Jakob disease (CJD), etc. These infectious particles, which accumulate in brain tissue during the incubation periods of TSEs, are comprised of abnormal folding conformations of a normal, ubiquitous protein called the "cellular" *prion protein* (PrP^{C}). This normal cellular prion protein is synthesized and eventually degraded through normal metabolic processes. In certain instances, however, protein molecules may become misfolded. When prion proteins misfold (hereafter called "prions"), the resultant shape change serves as a template to induce the misfolding of the normal cellular prion protein to produce more infectious prions and, thereby, propagate an infection. These abnormal prions resist proteosomal degradation and slowly accumulate in the brain and infect brain tissue.

The first described prion disease was scrapie, and so such infectious proteins are often designated collectively as PrP^{Sc} in all TSEs. Some scientists prefer to use other terms such as PrP^{res}, PrP^{d}, PrP^{TSE}, PrP^{CWD}, and PrP^{CJD} to designate the specific, abnormal forms of PrP associated with specific prion diseases.

For purposes of this guidance, the terms "prions" and "TSE agents" are synonymous. These abnormal prion proteins, although somewhat variable, share properties that distinguish them from normal PrP^{C}: they are usually insoluble in non-denaturing detergent-salt solutions and relatively resistant to digestion with the enzyme proteinase K. These properties are attributed to misfolding of PrP^{C} to yield isoforms that are enriched in beta-sheet secondary structure. Many scientific experts have concluded that the abnormal prion proteins themselves are the self-replicating infectious agents causing TSEs (Prusiner 1982, 2004); however, there remain notable reservations, as outlined by Manuelidis (2007a, b).

1. Manuelidis L. 2007a. A 25 nm virion is the likely cause of transmissible spongiform encephalopathies. Journal of cellular biochemistry; 100(4):897-915.

2. Manuelidis L, Yu ZX, Barquero N, Mullins B. 2007b. Cells infected with scrapie and Creutzfeldt-Jakob disease agents produce intracellular 25-nm virus-like particles.

Proceedings of the National Academy of Sciences of the United States of America;104(6):1965-70.

3. Prusiner SB. 1982. Novel proteinaceous infectious particles cause scrapie. Science; 216:136-44.

4. Prusiner SB. 2004. Detecting mad cow disease. Scientific American; 291(1):86-93.

(b) Purpose. This guideline provides pesticide applicants and registrants guidance on test systems and performance standards that apply to pesticide products intended to reduce the infectivity of prions on inanimate, environmental surfaces (hereafter called “prion-related products”). EPA encourages pesticide applicants and registrants to follow this guidance and submit a draft test protocol to EPA for review prior to conducting any studies. EPA may revise this guidance over time, as needed, to reflect advances in available test methods and scientific knowledge pertaining to prions. Studies conducted under this guideline are to be completed under EPA’s Good Laboratory Practice regulations (40 CFR Part 160).

(c) Guidance—

(1) Labeling Claims. Testing conducted according to currently available test methods are adequate for measuring reduction in prion infectivity, but not for demonstrating complete destruction or inactivation of prions. Accordingly, claims such as “inactivates,” “destroys,” “denatures” and “eliminates” are not supported by currently available test methods. Further, a prion-related claim should include the type of prion against which the product has been successfully tested. Assuming acceptable data were available, the following is the general format for a claim that EPA may consider accepting:

> “Has been demonstrated to reduce infectivity of prions (TSE agents) by (X) logs (insert log reduction number supported by data) based on a bioassay of the (insert prion type) in (insert type of organism in which the prions were tested).”

An example of a claim that EPA could find to be acceptable depending on supporting data would be:

> “Has been demonstrated to reduce infectivity of prions (TSE agents) by six (6) logs based on a bioassay of the scrapie prion in transgenic mice.”

(2) Selecting a Test System. The test system should be appropriate to the uses that appear on the proposed label.

a. Carrier-based Method. If the intended uses of a product are for reducing the infectivity of prions on inanimate surfaces, then a carrier-based, animal bioassay should be used to measure the amount of prion infectivity reduction that is achieved by the product when used according to label directions. Examples of published, carrier-based test methods include:

1. Lemmer K, Mielke M, Kratzel C, Joncic M, Oezel M, Pauli G, Beekes M. Decontamination of surgical instruments from prions. II. In vivo findings with a model system for testing the removal of scrapie infectivity from steel surfaces. J Gen Virol. 2008 Jan;89(Pt 1):348-58.

2. Peretz D, Supattapone S, Giles K, Vergara J, Freyman Y, Lessard P, Safar JG, Glidden DV, McCulloch C, Nguyen H-OB, Scott M, DeArmond SJ, and Prusiner SB. 2006. Inactivation of prions by sodium dodecyl sulfate. J. Virol. 80:322-331.

3. Weissmann C, Enari M, Klohn PC, Rossi P, Flechsig E. 2002. Transmission of prions. J Infect Dis 186 Suppl 2:S157-65.

4. Zobeley E, Flechsig E, Cozzio A, Enari M, and Weissman C. 1999. Infectivity of scrapie prions bound to a stainless steel surface. Molecular Medicine 5:240-243.

b. Suspension-based Test Method. If the intended use of a product includes only treating liquids (e.g., liquid wastes), then a suspension-based, animal bioassay should be used to measure the amount of prion infectivity reduction that is achieved by the product when used according to label directions. An example of a published, suspension-based test method is:

1. Peretz D, Supattapone S, Giles K, Vergara J, Freyman Y, Lessard P, Safar JG, Glidden DV, McCulloch C, Nguyen H-OB, Scott M, DeArmond SJ, and Prusiner SB. 2006. Inactivation of prions by sodium dodecyl sulfate. J. Virol. 80:322-331.

(3) Methods of Estimating the Reduction of Prion Infectivity. Prion diseases are generally characterized by a long asymptomatic incubation period, followed by the rapid onset of symptoms, followed by death. The length of the incubation period is reproducible upon repeated passages of a given prion and is inversely proportional to the log of the infectious dose of that prion, although at both very high and very low concentrations of infectivity the relationship is no longer linear. Two methods of estimating the reduction of prion infectivity have been employed: **endpoint titration** and **incubation time interval assay**. The former method may be best suited to analyze suspension-based testing and the latter may be best suited for carrier-based testing. Further, incubation time interval assays are generally not quite as accurate as endpoint titration assays (0.5 log difference[1]), and may be less reliable for quantifying very low levels of infectivity.

a. End-point Titration. An end-point titration is a classical method of determining the titer of a sample (i.e., the concentration of the pathogen). To begin with, a

[1] Prusiner SB, Cochran SP, Downey DE, Groth DF. Determination of scrapie agent titer from incubation period measurements in hamsters. Adv. Exp. Med. Biol. 1981;134: 385-99.

Prusiner SB, Cochran SP, Groth DF, Downey DE, Bowman KA, Martinez HM. Measurement of the scrapie agent using an incubation time interval assay. Ann. Neurol. 1982 Apr;11(4):353-8.

sample is serially diluted by a factor of 10 until less than 1 ID_{50} (the infectious dose which kills 50% of the pathogen) remains in the final dilution. The resulting set of serial dilutions is used to inoculate a corresponding set of experimental animals, typically at least four animals per 10-fold dilution. The experimental animals are observed for the appearance of symptoms, and the symptoms are scored. The animals are observed for a predetermined period, usually 450 days for hamsters or 500 days for mice. At the end of the predetermined period, any surviving animals would be humanely euthanized before they would die of the prion disease and given a thorough neuropathological examination that looks for signs of prion disease as described in section (4) below. The titer may be determined by several statistical methods (see references below). The titers of subsequent samples are determined in the same fashion.

The following are examples of end-point titration methods:

1. Andersen J, Barrett T, Scott GR. 1996. Appendix 3. Fifty percent effective dose (ED_{50}): Spearman-Kärber method. In Manual of the Diagnosis of Rinderpest. (FAO Animal Health Manual – 1) Food and Agriculture Organization of the United Nations. Rome. Available on-line: http://www.fao.org/docrep/w0049e/w0049e07.htm#appendix%203.%20fifty%20percent%20effective%20dose%20(ed50):%20spearman%20kärber%20method

2. Reed LJ and Muench H. A simple method for estimating fifty percent endpoints. Amer J Hyg 1938;27:493-7.

3. Bliss CI. The method of probits. Science. 1934 Jan 12;79(2037):38-39.

b. Incubation Time Interval Assay. An incubation time interval assay exploits the relationship between the length of the incubation period and the titer of the pathogen in an inoculum. An endpoint titration of a starting brain homogenate is used to establish an empirical relationship between prion titer and length of incubation period. Once the relationship is established, the titer of any subsequent sample is determined by observing the incubation period of an inoculated animal group (usually at least four) and using this value to predict a corresponding titer based on the empirically determined relationship. The initial endpoint titration should not be greatly, if at all, separated in time from the experimental assay. If the initial endpoint titration is to be replaced with historical animal model data, then sufficient control animals should be included to ensure that the historical animal model data are relevant to the experimental assay.

The following are examples of incubation time interval assay methods:

1. Prusiner SB, Cochran SP, Groth DF, Downey DE, Bowman KA, Martinez HM. 1982. Measurement of the scrapie agent using an incubation time interval assay. Ann. Neurol. April;11(4):353-8.

2. Prusiner SB, Cochran SP, Downey DE, Groth DF. 1981. Determination of scrapie agent titer from incubation period measurements in hamsters. Adv Exp Med Biol. 134:385-99.

The endpoint titration and incubation time interval assays yield similar results; however, the endpoint titration requires many more animals. As a result, the incubation time interval assay is the more widely used assay method for prions since it is faster, uses fewer animals, and provides results that are negligibly different (± 0.5 log)[2] compared to the endpoint titration. However, an endpoint titration assay may be more useful than an incubation time assay if the difference between the titer of the prions in the starting inoculum and the treated carrier is very high or very low, or if one wants to directly compare two samples with similar titers. A comparison of the number of animals required for these assays is listed in Appendix 1. The 263K strain of hamster-adapted scrapie is being provided as an example. The endpoint titration requires between two and four times the animals as the incubation time interval assay.

c. Other Relevant Tests. EPA welcomes data from screening tests (such as Western blot, ELISA, Protein Misfolding Cyclic Amplification (PMCA), or cell culture assays), but such tests are not a substitute for the suspension-based or carrier-based tests.

(4) Other important aspects of the test system include the following:

***Titer of prions*:** Depending on the test method and type of prion used, the titer of the prions in the initial inoculum may range from 10^4 to 10^{11} ID_{50} units/g of brain homogenate, based on currently available studies. After dilution and drying of the inoculum on the carrier or in a suspension, the titer of the prions may be further reduced by 1-2 logs. The study should have a reliable method for determining the concentration of prions in the initial inoculum and the concentration of prions dried on the carrier or mixed in a suspension. The dynamic range of the test method's ability to measure reduction in infectivity should be well established. In order for the test to be able to measure at least a six (6) log reduction in infectivity [see section (5) Evaluation of Success below], the titer of the prions on the carrier or in a suspension (i.e., the titer to which test animals will be exposed) should be at least 10^7 ID_{50} units/g brain homogenate (or about 10^9 ID_{50} units/g brain homogenate in the initial inoculum). Finally, the protocol should address and balance such issues as the preparation of the initial inoculum, the order in which animals are inoculated, and how the animals are housed (see Animal Housing below).

Prion type: Several types of prions are available for use in infectivity tests, such as scrapie prions (hamster adapted 263K), BSE prions (mouse adapted 301V, 310C and 6BP1), and sCJD prions (human). An <u>animal-related prion</u> (e.g., scrapie, CWD) should be selected for testing if the proposed use sites are <u>animal-related</u> (e.g., farm premises, farm equipment, veterinary clinics). A <u>human-related prion</u> (e.g., sCJD) should be selected if the proposed use sites are <u>related to humans</u> (e.g., surgical instruments, hospital rooms, laboratories). [Note: Prospective registrants should consult with the U.S. Food and Drug Administration (FDA) for uses that are also under its jurisdiction (e.g., medical

[2] See references in footnote 1.

devices or adjuncts to medical devices). The FDA considers claims of activity against prions (TSE agents) to be unique and unclassified, and recommends that product manufacturers seeking a product claim of reducing the infectivity of prions (TSE agents) for any healthcare use meet with FDA to learn the FDA's recommendations for seeking product approval.] Human-derived TSE materials of a known titer currently are available at only a few research laboratories.

***Age of test animals at beginning of study*:** Animals should be old enough to tolerate intracerebral inoculation without excessive mortality, i.e., in the following age ranges: mice, 6-10 weeks old; hamsters, 5-8 weeks old; and guinea pigs, 5-7 weeks old. The study should continue up to the end of the animals' normal life span (the point at which normal mortality begins to increase significantly). Historical mortality data on the selected strain of test animals should be provided to substantiate the selection of these critical lifespan points.

***Number of animals*:** The numbers of animals in the treatment and control groups should be sized based on statistical validity, and the calculations supporting the proposed animal group sizes should be included. The number of animals typically used is between 4 and 24.

***Types/Species of animals*:** The type/species of animal is determined by the test method selected. The test animals are either genetically homogeneous mice or hamsters, or transgenic (genetically altered to express the prion protein—PrP—of the host animal) mice or hamsters. If the animals are transgenic, the test protocol should identify the source of the transgenic gene material and available information that characterizes it. Native species of prions may also be tested in relevant animal species.

***Animal housing and environmental conditions*:** The number of animals per cage should be kept to a minimum and the cages should be compatible with the animals. Other animal housing issues should be addressed to avoid influencing the test results; for example, such as which groups may be housed next to each other, and rotating cages to balance exposure to heat, light, noise, etc. Transgenic or wild-type mice can be housed 5-8 per cage, depending on the size of the cage, and hamsters are generally housed two per cage. The temperature in the experimental animal room should be 22°C (± 3°C). The relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning. Lighting should be artificial, the sequence being 12 hours light and 12 hours dark.

***Length of study*:** The study should be extended as long as possible, taking into account the historical mortality data for the particular animal type/species used. Transgenic or wild-type mice should be kept for 500 days. Hamsters should be kept for 450 days.

Examination of animals at end of study or when animals die prematurely: When animals reach the defined age limit or die prematurely, tests should be performed on all members of both the treated and control groups, in order to ascertain whether they are infected with prions. Such tests should include at least one of the following: Western blot, immunological histochemistry or “blind passage.” The method for dealing with premature deaths and how to place them into the statistical calculations should also be addressed in the statistical evaluation plan for the study.

Evaluation of Success: The target criterion for success is no less than six (6) logs of reduction of infectivity in the treated versus untreated (control) groups.

Submission of Draft Protocol: Due to the wide variety of available animal bioassays for measuring reduction of prion infectivity by a prion-related product on environmental surfaces, the Agency strongly recommends that the registrant submit a draft test protocol to EPA for review prior to conducting such a study. The Agency also recommends that the registrant request a pre-registration conference to discuss registration data and labeling requirements for a prion-related product.

Appendix 1. Animals used in an endpoint titration versus an incubation time interval assay under different sets of assumptions

Assuming:

- Initial brain titer of 10^{10} ID_{50}/g
- 10 treatments are evaluated.
- 6 animals are used per log dilution.
- Each treatment reduces infectivity by 10^4.

Endpoint titration:

Initial titration (10 log dilutions)	10 x 6 = 60
10 x Titration of each treatment (7 log dilutions)	10 x 7 x 6 = 420
Total	480

Incubation time interval assay:

Titration/calibration curve (10 log dilutions)	10 x 6 = 60
10 x 1 group per treatment	10 x 6 = 60
Total	120

Same assumptions except:

- Each treatment reduces infectivity by 10^6.

Endpoint titration:

Initial titration (10 log dilutions)	10 x 6 = 60
10 x Titration of each treatment (5 log dilutions)	10 x 5 x 6 = 300
Total	360

Incubation time interval assay:

Titration/calibration curve (10 log dilutions)	10 x 6 = 60
10 x 1 group per treatment	10 x 6 = 60
Total	120

Same assumptions except:

- Each treatment reduces infectivity by 10^8.

Endpoint titration:

Initial titration (10 log dilutions)	10 x 6 = 60
10 x Titration of each treatment (3 log dilutions)	10 x 3 x 6 = 180
Total	240

Incubation time interval assay:

Titration/calibration curve (10 log dilutions)	10 x 6 = 60
10 x 1 group per treatment	10 x 6 = 60
Total	120